Aquaculture 479 (2017) 780–789

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Effect of winter feeding frequency on growth, survival, and fatty acid metabolism of juvenile bluegill (*Lepomis macrochirus*) and hybrid bluegill (L. *cyanellus* × *L. macrochirus*)

Luke A. Roy[a,*], Steven D. Rawles[b], Anita M. Kelly[a], Carl D. Webster[b], Nathan Stone[a], Alf Haukenes[a]

[a] Aquaculture/Fisheries Center, University of Arkansas at Pine Bluff, 1200 North University Drive, Pine Bluff, AR 71601, United States
[b] Harry K. Dupree Stuttgart National Aquaculture Research Center, PO Box 1050, 2955 Highway 130 East, Stuttgart, AR 72160, United States

ARTICLE INFO

Keywords:
Fatty acids
Winter mortality
Canonical discriminant analysis
Bluegill
Hybrid bluegill

ABSTRACT

There has been an increasing global demand for large bluegill (*Lepomis macrochirus*) and its hybrid (female *Lepomis cyanellus* × male L. *macrochirus*) to supply the food-fish market. However, production of market-size bluegill requires producers to overwinter them in temperate regions. Winter fish mortality is widely cited by fish farmers in temperate regions of the U.S. as a major factor that decreases profitability and sustainability. We evaluated the effect of three different feeding frequencies on survival, weight loss, and fatty acid composition of two species of Centrarchids held at a constant low temperature (7–9 °C) to simulate winter conditions. Bluegill (1.46 ± 0.06 g) and hybrid bluegill (2.58 ± 0.22 g) were stocked in separate 603-L recirculating systems configured with 9 tanks each (3 replicate tanks/feeding regime) and fed either twice per week, once per week, or once per month for 13 weeks. There were no differences among feeding regimes in final fish weight, survival, weight loss as a percent of initial weight, condition factor, and SGR for either taxon of fish. Survival was high among all treatments (89–98%). Regardless of feeding regime, all fish lost weight. Hybrid bluegill lost less weight (12–17% of initial body weight) than bluegill (18–20%). The concentrations and composition of fatty acids also changed markedly in response to feeding frequency according to canonical discriminant analysis. Fatty acid profiles among initial vs. post-winter bluegills fed at different frequencies were indicative of severe deficiencies in n-6 and n-3 essential fatty acids and preservation of long chain polyunsaturated fatty acids, especially when fish were fed less than twice per week. In conclusion, significant weight loss and reductions in key fatty acids needed for energetic needs were observed in both native and hybrid bluegill, regardless of feeding regime, indicating that the feeding rates examined in this study did not prove beneficial at 7–9 °C in preserving winter robustness.

1. Introduction

There has been an increasing global demand for large bluegill (*Lepomis macrochirus*) and its hybrid (female *Lepomis cyanellus* × male L. *macrochirus*) to supply the food-fish market. This is due to bluegill possessing several characteristics that make them desirable for aquaculture that include rapid growth, acceptance and efficient utilization of prepared diets (Tidwell et al., 1992), and an aggressive nature that makes them attractive to anglers (Brunson and Robinette, 1986). The highest specific growth rate for juvenile bluegill has been reported to occur at 30 °C (optimal temperature range between 26 and 32 °C; Lemke, 1977); however, production of market-size bluegill in the temperate climate of the southeastern United States still requires overwintering.

Winter fish mortality is a phenomenon that has been widely cited by fish farmers in temperate regions of the U.S. as a major factor that decreases profitability and sustainability. As water temperatures decline during the winter months, warmwater fish do not feed as aggressively, or at all. Feeding strategies to reduce mortality and minimize weight loss during this period vary widely in temperate regions of the U.S. Some producers may decrease feeding frequency as temperatures fall toward winter, cease feeding altogether when water temperatures fall below a certain level, or feed intermittently during the winter (Brunson and Robinette, 1986; Webster et al., 1992; Goodgame-Tiu et al., 1994; Rowan and Stone, 1994; McNulty et al., 2000; Nanninga et al., 2011). Currently, there is no generally accepted

* Corresponding author at: School of Fisheries, Aquaculture & Aquatic Sciences, Auburn University, Auburn, AL 36849, United States.
E-mail address: royluke@auburn.edu (L.A. Roy).

http://dx.doi.org/10.1016/j.aquaculture.2017.07.026
Received 7 November 2016; Received in revised form 17 July 2017; Accepted 18 July 2017
Available online 18 July 2017

protocol practiced industry wide with respect to feeding practices. As feed consumption declines, however, fish weight loss occurs as the metabolism of fish is directly correlated to water temperature, i.e., both routine metabolic rate and metabolic scope, which measure capacity for physiological performance, are significantly depressed in warm water fish with declining temperature (Fontaine et al., 2007). While general culture techniques have made important advances in the production of large bluegill (Tidwell et al., 1994; Hicks et al., 2009; Wang et al., 2009) and nutritional requirements as well as diet formulations have been more clearly defined (Tidwell et al., 1992; Tidwell and Webster, 1993; Hoagland et al., 2003; Twibell et al., 2003; Masagounder et al., 2011; Yang et al., 2016), there are no published data on the effects of different feeding regimens on growth, body composition and health of bluegill. Nevertheless, winter mortality appears to be a universal problem among Centrarchid producers, in spite of a wide range of feeding practices.

One hypothesis suggests that fatty acid composition of overwintering bluegill might impact winter survival through the strong role essential fatty acids (EFAs) play in membrane fluidity, immunity and disease resistance (Tocher and Glencross, 2015). Inadequate levels of EFA can result in growth retardation, fin erosion, myocarditis, increased sensitivity to stress, shock syndrome and death (Castell et al., 1972; Watanabe, 1982). One adaptation to cold temperature is to increase the proportion of long-chain polyunsaturated fatty acids (LC-PUFA) and reduce the proportion of saturated fatty acids in cell membrane phospholipids in order to increase membrane fluidity at lower temperature (Farkas et al., 2001; Tocher, 2003). Any changes in cell membrane fatty acid profiles also alter functions that affect the immune system (Verlhac Trichet, 2010). Reduced availability of LC-PUFA, e.g., EPA and DHA, has been associated with chronic elevation of plasma cortisol, which inhibits immune responses through blocking macrophage cytokine production, thus affecting disease resistance (Montero and Izquierdo, 2010).

Nevertheless, studies on the fatty acid composition of bluegill in regards to environment, diet, or health are extremely rare. Rude et al. (2016) found that LC-PUFA levels were quite substantial but variable in wild caught bluegill from the Illinois River system and ranged from 15 to 30%, depending on the location fish were taken; their data suggest that fatty acid (FA) profiles can infer habitat use and habitat-specific foraging of fishes in large river-floodplain ecosystems. Glass et al. (1974) noted changes in the content of furan fatty acids in liver and testes of bluegill in response to spawning season. Kelly et al. (1958) found no significant change in the PUFA profile of bluegill captured from the wild and those subsequently fed a low-fat or cottonseed oil diet over 60 days, whereas in those fed a menhaden oil diet, the fatty acid composition changed to resemble the composition of the oil. Hence, bluegill may be able to synthesize PUFAs, though not necessarily essential fatty acids, from non-fatty precursors. This is particularly relevant since dietary intake or elongation/desaturation of fatty acids are the two major means of altering PUFA levels in fish (Tocher and Glencross, 2015). If bluegill have limited ability to elongate/desaturate essential PUFA in order to increase membrane fluidity for cold climate, then these essential fatty acids must be ingested (Hazel and Livermore, 1990). In contrast to potential connections between feed intake or feed composition and winter survival of bluegill, Fontaine et al. (2007) suggested that metabolic capacity in warm water fish tends to limit performance at lower temperatures rather than feed composition. Therefore, the objectives of this trial were to evaluate the effect of three different feeding regimes on survival, weight loss and fatty acid composition of two species of Centrarchids held at a constant low temperature to simulate winter conditions.

2. Materials and methods

2.1. Fish and culture system

Bluegill and hybrid bluegill were obtained from J.M. Malone and Son (Lonoke, AR, USA) and transported to the University of Arkansas at Pine Bluff Lonoke Fish Disease Diagnostic Laboratory (Lonoke, AR, USA) in November 2013. Fish were held in separate 150-L holding tanks connected to a larger recirculating system. The fish were held for approximately 6 weeks prior to the commencement of experimental trials to determine that they were disease free and fully acclimated to laboratory conditions. Fish were fed daily ad libitum a 36% protein commercial diet (Delta Western, Indianola, MS, USA) during the holding period. When the trial was initiated, acclimated bluegill (1.46 ± 0.06 g), and hybrid bluegill (2.58 ± 0.22 g) were stocked in separate 603-L recirculating systems as described below and initial samples of whole fish were collected at this time and frozen at − 20 °C until analysis for initial fatty acid profiles.

Each system was configured with 9 tanks (3 replicate tanks per feeding regime) and a sump equipped with a biofilter. Flow rate in each tank was set at 2.4 L min^{-1}. Following stocking of each system (15 fish per tank for a total of 135 fish per system), temperature was reduced from ambient (22 °C) to a target temperature of 8 °C over a period of 7 days. Water temperatures in the culture systems were maintained by using drop-in chillers (0.19 KW) with digital temperature controllers (TradeWind Chillers, Escondido, CA, USA). Water chillers were the same model, and water volumes were nearly identical in the two experimental systems. However, the capacity of individual chillers to maintain target temperatures varied somewhat resulting in slight temperatures differences among systems. Temperature was monitored twice daily (morning and evening) and adjusted as needed. Mean water temperatures were maintained between 7.0 and 9.2 °C in all trials.

Total ammonia nitrogen, dissolved oxygen and pH remained within acceptable limits. Water quality (mean ± standard deviation) was acceptable for the culture of both species. In the native bluegill trial dissolved oxygen (9.41 ± 0.80 mg L^{-1}), temperature (7.00 ± 0.60 °C), pH (8.10 ± 0.20), total ammonia nitrogen (0.31 ± 0.11 mg L^{-1}), and nitrite nitrogen (0.00 ± 0.00 mg L^{-1}) remained within acceptable limits. In the hybrid bluegill trial dissolved oxygen (9.09 ± 0.83 mg L^{-1}), temperature (8.00 ± 1.25 °C), pH (8.14 ± 0.21), total ammonia nitrogen (0.32 ± 0.13 mg L^{-1}) also remained within acceptable limits. Nitrite nitrogen (0.31 ± 0.34 mg L^{-1}) was slightly high but did not appear to negatively impact the fish. There was a 3-week spike in nitrite-nitrogen (1.5 mg L^{-1}) in the hybrid bluegill system, however no mortalities occurred as a result. When the spike occurred, sodium chloride was added (0.25 g L^{-1}) to the system to raise chloride levels.

2.2. Feeding regimes and sampling

Bluegill and hybrid bluegill were fed ad libitum the same commercial diet (Table 1) offered during the experimental period using three different feeding regimes. Feeding regimes consisted of either twice per week (2 ×/wk), once per week (1 ×/wk), or once per month (1 ×/mo). These feeding regimes were determined based on the range of current winter feeding practices of commercial bluegill and hybrid bluegill farmers in Arkansas. Both taxa consumed feed during feeding events throughout the trial, albeit very little in some cases. Fish were given 5–10 min to feed. If feeding ceased no further feed was offered on that day. Excess feed was not removed from the tanks. Following 13 weeks of culture at low temperature, the trials were terminated to assess weight loss, survival, specific growth rate and Fulton condition factor. Weight loss, specific growth rate (SGR) and Fulton condition factor (K) were calculated as follows:

$$\text{Weight loss } (\%) = 100\,(W_f - W_i)/W_i,$$

Table 1
Composition (dry weight basis) of a commercial diet used in a winter feeding study of juvenile (< 1.5 g) native and hybrid bluegill.

Nutrients	Composition
Protein (%)	38.6
Lipid (%)	6.1
Dry matter (%)	93.1
Energy (cal/g)	4468
Fatty acids	%
14:0	1.90
16:0	10.40
17:0	1.29
18:0	5.09
Sum of saturates	*18.68*
16:1	2.98
18:1n–7 (VAC)[a]	2.90
18:1n–9	15.20
20:1	4.89
Sum of monoenes	*25.97*
18:2n–6 (LA)[b]	10.80
18:3n–3 (ALA)[c]	8.11
20:3 (all)	10.50
20:5n–3 (EPA)[d]	6.37
22:6n–3 (DHA)[e]	9.62
	Totals
PUFA[f]	*45.40*
Σ n-3[g]	*24.10*
Σ n-6[h]	*9.62*
n-6/n-3[i]	*0.40*

[a] Cis-vaccenic acid (VAC).
[b] Linoleic acid (LA).
[c] Alpha-linolenic acid (ALA).
[d] Eicosapentaenoic acid (EPA).
[e] Docosahexaenoic acid (DHA).
[f] Polyunsaturated fatty acids (PUFA).
[g] Sum of the n-3 fatty acids.
[h] Sum of the n-6 fatty acids.
[i] Ratio of the total n-6 fatty acids. Divided by the total n-3 fatty acid.

where W_f and W_i are final and initial fish weight (g).

$$SGR\ (\%\cdot d^{-1}) = 100\ (\ln\ W_f - \ln\ W_i)/t,$$

where t is time (d) fed.

$$K = 100\ (W_f/L_f^3),$$

where, L_f is final fish length (mm).

2.3. Lipid and fatty acid analysis

Whole fish were collected for fatty acid analysis both at the start and end of the trial and stored frozen at − 20 °C until analysis. Fish were weighed whole and lyophilized (Labconco Triad model 7400040 freeze dryer, Kansas City, MO, USA), then ground to a homogenous state in a laboratory blender (Waring, Inc., New Hartford, CN, USA). Lipid was extracted from the lyophilized tissues using the Folch method (Folch et al., 1957). Fatty acids (FAs) were saponified and dervatized to FA methyl esters (FAMEs) for gas chromatography flame ionization detection (GC-FID) as follows: to each tube of extracted lipid, 100 μL of a tridecanoic acid (C13:0) standard solution (Nu-Chek-Prep, Inc., N-13A-A7-N, Elysian, MN, USA) in hexanes was added as the internal standard and evaporated to dryness using a nitrogen evaporator (N-Evap, Inc., Berlin, MA, USA). Subsequently, 1 mL of 0.5 N potassium hydroxide (KOH) in methanol was added and tubes were flushed with nitrogen, capped, and placed in a 70 °C water bath for 10 min in order to saponify FAs in the extracts. Tubes were removed from the bath and allowed to cool, after which 1 mL of 14% boron trifluoride (BF_3) in methanol was added. Tubes were flushed with nitrogen, capped, and placed in a 70 °C water bath for an additional 30 min. FAME samples were then removed and allowed to completely cool. To each tube 2 mL of hexane and 2 mL of saturated NaCl solution was added. Tubes were flushed with nitrogen, capped, vortexed, and allowed to stand until phase separation. The hexane (upper) layer was pipetted into a second vial containing sodium sulfite (Na_2SO_4) to remove residual moisture, then transferred to gas chromatography (GC) vials for analysis.

FAMES were analyzed using a Varian GC-FID model CP3800 (Varian, Inc., Walnut Creek, CA, USA) equipped with a capillary column (100 m, 0.25 mm; Varian CP for FAME #CP7420) using helium as the carrier gas. Injector port and detector temperatures were maintained at 250 °C. Samples containing FAMEs were injected into the column in 1 μL of hexane using an autosampler (Varian model 8200). Flow rates were constant throughout the run (hydrogen - 30 mL·min^{-1}; air - 300 mL·min^{-1}; and makeup gas - 31.7 mL·min^{-1}). Column oven temperature was initially 100 °C held for 10 min, then increased to 160 °C at a rate of 15 °C·min^{-1} and held for 10 min; finally, temperature increased to 250 °C at a rate of 2.5 °C·min^{-1} and held for 10 min. Sample FAMES were identified and quantified by comparing peak retention times and area counts to those of serially diluted mixtures of FAME reference standards (GLC-473 + C16:1TΔ9, C21:0, and C23:0 methyl esters; Nu-Chek-Prep, Inc., Elysian, MN, USA). As previously described, C13:0 was added prior to methylation and esterification to serve as the internal standard.

2.4. Statistical analysis

Growth and survival data were analyzed using one-way ANOVA and Student Newman Keuls multiple range test in SAS version 9.3 (SAS Institute, Cary, NC, USA) to determine if significant differences ($P < 0.05$) existed among treatment means (Ott, 1977) of individual responses in bluegill and hybrid bluegill. Fatty acid profiles were subjected to multivariate analysis of variance (MANOVA) followed by stepwise discriminant (SDA) analysis using the SAS 9.3 program STEPDISC (SAS Institute, Cary, NC). For each taxon (native vs. hybrid bluegill), SDA was used to optimize the number of fatty acids in the profile required to discriminate among the following fish classes: initial (i) fish, fish fed once per month (OPM; M), fish fed once per week (OPW; 1), or fish fed twice per week (TPW; 2) when held at 7–9 °C. The fatty acids and their abundances selected by SDA were then subjected to canonical discriminant analysis (CDA) using the SAS program CANDISC (SAS Institue, Cary, North Carolina) to test the null hypotheses that fatty acid profiles did not differ among initial fish and fish fed at different frequencies and to indicate the relative contribution of each fatty acid to class discrimination (Johnson and Wichern, 2002). A plot of the first two canonical variates (*Can1*, *Can2*) was used to visually assess differences in FA profiles among fish treatments. Pooled within-treatment standardized canonical coefficients for each canonical variate were used to indicate the degree of correlation between fatty acids and the canonical variate that maximally separated fish classes. The canonical coefficients for each fatty acid included in the model were then ordered to determine trends in composition among the four fish classes in each species (Hair et al., 2005). The above procedure was repeated after excluding initial fish profiles in order to further discriminate among fed fish only. Univariate ANOVA and Tukey-Kramer multiple comparisons technique were employed to further investigate differences among fish classes for individual fatty acids found significantly discriminatory in the SDA. Fatty acid abundances were log transformed prior to discriminant analyses. For comparison of initial and fed fish FA profiles, Pillai's trace statistic was used to indicate the significance of the MANOVA, whereas Wilks lambda was used for comparisons among FA profiles of fed fish alone. A probability level of < 0.05 was considered significant for statistical comparisons.

Table 2
Initial weight (g), final weight (g), survival (%), weight loss (% of initial weight), Fulton condition factor (K; g·mm^{-3}), and specific growth rate (SGR; %·d^{-1}) of native and hybrid bluegill fed different feeding regimens at low temperature (7–9 °C) for 13 weeks. Values represent mean ± standard deviation. Values with different subscripts were significantly different ($P \leq 0.05$).

Feeding regimen	Initial weight (g)	Final weight (g)	Survival (%)	Weight loss (%)	K (g·mm^{-3})	SGR (%·d^{-1})
	Native bluegill					
2 ×/week	1.42 ± 0.03	1.16 ± 0.03	97.8 ± 3.87	18.42 ± 2.16	1.11 ± 0.02	−0.217 ± 0.028
1 ×/week	1.48 ± 0.09	1.22 ± 0.12	88.9 ± 10.2	18.06 ± 3.74	1.11 ± 0.05	−0.213 ± 0.048
1 ×/month	1.47 ± 0.02	1.18 ± 0.01	95.6 ± 7.68	20.03 ± 0.62	1.13 ± 0.03	−0.238 ± 0.009
Pr > F	*0.422*	*0.615*	*0.397*	*0.617*	*0.811*	*0.615*
	Hybrid bluegill					
2 ×/week	2.63 ± 0.25	2.31 ± 0.12	95.6 ± 7.68	12.19 ± 4.72	1.22 ± 0.03	−0.139 ± 0.058
1 ×/week	2.57 ± 0.27	2.13 ± 0.11	93.3 ± 0.00	16.86 ± 5.78	1.14 ± 0.03	−0.198 ± 0.075
1 ×/month	2.52 ± 0.23	2.13 ± 0.07	88.9 ± 7.68	15.12 ± 6.23	1.15 0.06	−0.176 ± 0.080
Pr > F	*0.858*	*0.119*	*0.460*	*0.613*	*0.139*	*0.615*

3. Results

3.1. Performance measures

Following 13 weeks of culture, there were no differences ($P > 0.05$) among feeding regimes in final fish weight, survival, weight loss as a percent of initial weight, K, or SGR (Table 3) for either taxon of fish. Survival was high among all treatment combinations and ranged from 89 to 98%. Regardless of feeding regime, all fish lost weight during the 13-week trial (Table 2). Hybrid bluegill lost less weight (12.2–16.9% of initial body weight) than bluegill (18.1–20.0%).

3.2. Fatty acids profiles

Fatty acid profiles of pre-winter (initial) native bluegill were significantly different than those of post-winter fed bluegill (Table 3). With the exception of pentadecylic acid, 15:0, saturates tended to be higher in initial fish than post-winter fed native bluegill. Monoenes tended to be lower in initial bluegill than in post-winter fed bluegill, with the exception of cis-vaccenic acid (VAC; 18:1n-7) which was higher in the initial fish. Concentrations of linoleic acid (LA; 18:2n-6) were lower in post-winter fed bluegill than in the initial fish, whereas concentrations of arachidonic (ARA; 20:4n-6), eicosapentaenoic (EPA; 20:5n-3), adrenic (22:4n-6), and docosapentaenoic (DPA, 22:5n-3) acids were lower in the initial fish compared to concentrations found in post-winter fed fish. Although the differences were not statistically significant ($P = 0.174$), docosahexaenoic acid (DHA; 22:6n-3) levels also were numerically lower (6.8%) in the initial fish compared to mean levels found in post-winter fed native bluegill (7.5–8.7%).

Fatty acid profiles of initial hybrid bluegill also differed from those of post-winter fed hybrid bluegill (Table 4). Saturates of 14 to 17 carbons tended to be higher in initial fish; whereas, saturates of 18 to 23 carbons tended to be lower in the initial fish when compared to post-winter fed hybrid bluegill. With the exception of nervonic acid (24:1n-9), concentrations of monoenes were higher in the initial fish than in post-winter fed hybrid bluegill. Similar to native bluegill, levels of cis-vaccenic acid (18:1n-7) in initial hybrid bluegill were higher than in post-winter fed hybrid bluegill. Concentrations of LA in initial samples of hybrid bluegill were intermediate to levels found among post-winter fish fed different regimes. Similar to native bluegill, concentrations of ARA, EPA; adrenic acid, DPA, and DHA were significantly lower in the initial hybrids compared to concentrations found in post-winter fed hybrids.

Multivariate analysis of variance (MANOVA) of fatty acid abundances in native (Pillai's trace $F = 6.6$; df = 9, 60; $P \leq 0.0001$) and hybrid (Pillai's trace $F = 11.4$; df = 27, 42; $P \leq 0.0001$) bluegill found that the initial and fed bluegills possessed significantly different FA profiles (Fig. 1). Three of the total 29 fatty acids measured were sufficient to discriminate between initial native bluegill vs. fed native bluegill according to SDA/CDA (Table 6; left columns), whereas trends in nine fatty acids were capable of differentiating among initial and fed hybrid bluegill (Table 5; right columns).

Table 3
Whole-body fatty acid composition (%) of initial native bluegill and those fed one of three feeding regimens at low temperature (7–9 °C) for 13 weeks. Values are least square means of four replicates and the pooled SE (SE*p*). Means in each row with different letters are significantly different ($P \leq 0.05$).

Fatty acid	Initial	2 ×/week	1 ×/week	1 ×/month	SE*p*	*Pr > F*
14:0	0.77	0.89	0.78	0.71	0.04	*0.335*
15:0	0.60 b	0.80 a	0.74 a	0.76 a	0.02	*0.002*
16:0	27.29 a	23.42 b	23.30 b	22.82 b	0.04	*0.010*
17:0	1.34	1.26	1.32	1.32	0.01	*0.164*
18:0	8.74 a	6.66 c	7.16 bc	7.46 b	0.02	*< 0.001*
19:0	0.63	0.79	0.74	0.77	0.03	*0.103*
20:0	0.55	0.59	0.54	0.54	0.01	*0.353*
23:0	1.12	1.86	1.30	1.33	0.07	*0.089*
Sum of saturates	*41.04*	*36.27*	*35.88*	*35.71*		
14:1	0.42	0.43	0.34	0.31	0.04	*0.376*
16:1	3.56 b	5.33 a	4.96 a	5.02 a	0.05	*< 0.001*
18:1n-11	0.56	0.64	0.61	0.62	0.02	*0.320*
18:1n–7 (VAC)[a]	2.95 b	3.16 ab	3.16 ab	3.30 a	0.02	*0.026*
18:1n–9	8.83 a	7.30 b	7.01 b	6.93 b	0.03	*< 0.001*
20:1	0.75 b	1.02 a	0.93 a	1.02 a	0.02	*< 0.001*
22:1	0.36	0.50	0.38	0.42	0.14	*0.970*
24:1	2.30	2.96	3.02	3.24	0.07	*0.056*
Sum of monoenes	*19.73*	*21.34*	*20.41*	*20.86*		
18:2n–6 (LA)[b]	8.80 a	5.33 b	5.17 b	4.79 b	0.05	*< 0.001*
20:2n–6	0.87	0.97	0.89	0.91	0.02	*0.475*
18:3n–3 (ALA)[c]	2.70	2.59	2.75	2.62	0.03	*0.602*
20:3 (all)	1.69	1.49	1.70	1.70	0.06	*0.621*
20:4n–6 (ARA)[d]	7.50 b	8.08 ab	8.67 ab	9.36 a	0.04	*0.020*
20:5n–3 (EPA)[e]	1.59 c	2.81 b	2.99 ab	3.18 a	0.02	*< 0.001*
22:3n–3	1.16	1.55	1.38	1.36	0.05	*0.104*
22:4n–6	1.05 b	1.39 a	1.33 a	1.42 a	0.03	*0.004*
22:5n–3 (DPA)[f]	1.70 b	2.55 a	2.51 a	2.72 a	0.04	*< 0.001*
22:6n–3 (DHA)[g]	6.82	7.54	7.87	8.71	0.07	*0.174*
	Totals					
PUFA[h]	*33.88*	*34.30*	*35.26*	*36.77*		
Σ n-3[i]	*13.97*	*17.04*	*17.50*	*18.59*		
Σ n-6[j]	*18.22*	*15.77*	*16.06*	*16.48*		
n-6/n-3[k]	*1.30*	*0.93*	*0.92*	*0.89*		

[a] Cis-vaccenic acid (VAC).
[b] Linoleic acid (LA).
[c] Alpha-linolenic acid (ALA).
[d] Arachidonic acid (ARA).
[e] Eicosapentaenoic acid (EPA).
[f] Docosapentaenoic acid (DPA).
[g] Docosahexaenoic acid (DHA).
[h] Polyunsaturated fatty acids (PUFA).
[i] Sum of the n-3 fatty acids.
[j] Sum of the n-6 fatty acids.
[k] Ratio of the total n-6 fatty acids divided by the total n-3 fatty acids.

Table 4
Whole-body fatty acid composition (%) of initial hybrid bluegill and those fed one of three feeding regimens at low temperature (7–9 °C) for 13 weeks. Values are least square means of four replicates and the pooled SE (SE*p*). Means in each row with different letters are significantly different ($P \leq 0.05$).

Fatty acid	Initial	2 ×/week	1 ×/week	1 ×/ month	SE*p*	*Pr > F*
14:0	2.99 a	1.12 b	0.51 c	0.79 bc	0.07	*< 0.001*
15:0	0.91 a	0.63 b	0.61 b	0.68 b	0.03	*0.005*
16:0	30.39	25.46	19.59	23.43	0.18	*0.113*
17:0	1.19 a	0.95 b	1.04 ab	1.13 ab	0.03	*0.035*
18:0	6.49 c	6.96 bc	8.24 a	7.73 ab	0.03	*< 0.001*
19:0	0.51 b	0.64 ab	0.68 a	0.76 a	0.03	*0.003*
20:0	0.44 c	0.70 a	0.56 b	0.56 b	0.01	*< 0.001*
23:0	0.90 b	1.19 ab	1.19 ab	1.36 a	0.04	*0.007*
Sum of Saturates	*43.82*	*37.65*	*32.42*	*36.44*		
14:1	1.24 a	0.29 b	0.14 c	0.19 bc	0.03	*< 0.001*
16:1	7.40 a	5.11 b	4.65 b	5.15 b	0.05	*< 0.001*
18:1n–11	0.56	0.63	0.54	0.60	0.02	*0.327*
18:1n–7 (VAC)[a]	3.55 a	2.61 b	2.70 b	2.82 b	0.03	*< 0.001*
18:1n–9	12.52 a	8.48 b	7.98 b	7.66 b	0.06	*< 0.001*
20:1	1.34	1.32	1.29	1.19	0.03	*0.553*
22:1	1.01	0.96	0.79	0.83	0.09	*0.670*
24:1n–9	1.35 b	2.42 a	3.17 a	3.10 a	0.10	*0.001*
Sum of Monoenes	*28.97*	*21.82*	*21.26*	*21.54*		
18:2n–6 (LA)[b]	7.65 bc	10.54 a	8.53 ab	6.49 c	0.06	*< 0.001*
18:3n–3 (ALA)[c]	2.13 a	1.77 b	1.71 b	1.86 ab	0.03	*0.009*
20:2n–6	0.65 b	0.98 a	1.07 a	1.02 a	0.03	*< 0.001*
20:3	1.09 b	1.73 a	1.75 a	1.76 a	0.05	*0.002*
20:4n–6 (ARA)[d]	3.07 c	6.13 b	8.73 a	8.39 a	0.06	*< 0.001*
20:5n–3 (EPA)[e]	1.09 c	1.75 b	2.13 ab	2.24 a	0.04	*< 0.001*
22:3n–3	0.67 b	1.27 a	1.23 a	1.47 a	0.06	*0.001*
22:4n–6	0.68 b	1.03 a	1.25 a	1.27 a	0.04	*< 0.001*
22:5n–3 (DPA)[f]	1.09 b	1.71 a	2.15 a	2.00 a	0.05	*< 0.001*
22:6n–3 (DHA)[g]	2.81 c	7.09 b	11.09 a	8.25 ab	0.10	*< 0.001*
	Totals					
PUFA[h]	*20.93*	*34.00*	*39.64*	*34.75*		
Σ n-3[i]	*7.79*	*13.59*	*18.31*	*15.82*		
Σ n-6[j]	*12.05*	*18.68*	*19.58*	*17.17*		
n-6/n-3[k]	*1.55*	*1.37*	*1.07*	*1.09*		

[a] Cis-vaccenic acid (VAC).
[b] Linoleic acid (LA).
[c] Alpha-linolenic acid (ALA).
[d] Arachidonic acid (ARA).
[e] Eicosapentaenoic acid (EPA).
[f] Docosapentaenoic acid (DPA).
[g] Docosahexaenoic acid (DHA).
[h] Polyunsaturated fatty acids (PUFA).
[i] Sum of the n-3 fatty acids.
[j] Sum of the n-6 fatty acids.
[k] Ratio of the total n-6 fatty acids divided by the total n-3 fatty acids.

For native bluegill (Table 5; left columns), the first canonical variate (*Can1*) accounted for 98% of the total variance among the data and maximally separated initial from fed fish (Fig. 1; left panel). Similarly, for hybrid bluegill (Table 5; right columns), *Can1* accounted for 90% of the total variance and also separated initial from fed fish (Fig. 1; right panel). The second canonical variate (Fig. 1; *Can2*) was unable to fully discriminate ($R^2 = 0.509$) among groups of fed native bluegill (m, 1, or 2) and explained only 2% of the data dispersion (Table 5; left columns). However in the hybrid bluegill fatty acid data, *Can2* explained an additional 9% of the variance and was better able ($R^2 = 0.971$) to distinguish among fed groups (Table 5; right columns). Loading scores, i.e., pooled within-class standardized canonical coefficients, for fatty acids in initial vs. fed native bluegill (Table 5; left columns) indicate that 16:1trans, 18:0, and 20:5 were all that were needed to fully distinguish between initial vs. fed native bluegill although other individual fatty acids were also statistically different among these groups (Table 3); moreover; 16:1trans and 20:5 were significantly lower and 18:0 was significantly higher in initial fish compared to fed native bluegill (Table 3).

Loading scores for fatty acids in initial vs. fed hybrid bluegill (Table 5; right columns) indicate that monoenes 14:1 and 16:1 along with 20:0, 20:2, 20:3, 20:4, and 22:5 discriminated among hybrid bluegill groups. Specifically, 14:1 and 16:1 were higher in the initial hybrid bluegill whereas 20:0, 20:2, 20:3, 20:4, 22:5 were higher in the fed hybrid bluegill (Table 4).

Fatty acid profiles of the two bluegill taxa also differed significantly by feeding frequency at 7–9 °C according to MANOVA (native bluegill: Wilks Lambda $F = 4.27$; df = 26, 6; $P \leq 0.039$; hybrid bluegill: Wilks Lambda $F = 23.4$; df = 12, 20; $P \leq 0.0001$) when initial fish were excluded from the analyses (Fig. 2). Thirteen fatty acids were able to discriminate between native bluegill subjected to different feeding frequencies according to SDA/CDA (Table 6; left columns), whereas trends in five fatty acids were capable of differentiating among groups of fed hybrid bluegill (Table 6; right columns).

For native bluegill (Table 6; left columns), *Can1* accounted for 95% of the total variance and maximally separated fish fed twice per week from those fed less frequently (Fig. 2; left panel). *Can2* accounted for an additional 5% of the variance and attempted to separate fish fed once per week (1) from those fed once per month (m). For hybrid bluegill (Table 6; right columns), *Can1* accounted for 83% and *Can2* accounted for additional 17% of the total variance while maximally separating all three groups of fed fish (Fig. 2; right panel).

Among fed native bluegill, 20:4, 22:4, 22:5 and 22:6 were highly discriminatory (Table 6; left columns) based on the absolute values of the loading scores (Table 6; left columns). Concentrations of stearate (18:0), ARA (20:4), EPA (20:5), 22:4, DPA (22:5) and DHA (22:6) tended to increase with decreasing feeding frequency (Table 3). Among groups of fed hybrid bluegill, the lack of a large difference among absolute values of loading scores (Table 6; right panel) for the five significant fatty acids in the profiles suggests no fatty acid stood out in terms of discriminatory power. The saturates 14:0 and 20:0 were higher in hybrid bluegill fed twice per week compared to those fed less frequently. Linoleic acid (18:2n-6) decreased with decreasing feeding frequency (Table 4); whereas, 19:0 and 20:5 slightly increased with decreasing feeding frequency (Table 4).

4. Discussion

The working hypothesis in the current study was that winter mortality in Arkansas Centrarchid production is associated with reduced feeding and resulting detrimental changes to essential fatty acid depots available for adaptation to cold, disease resistance, and immunity. Regardless of feeding frequency, all fish lost weight in the current experiment when water temperatures were maintained at 7–9 °C for 13 weeks, but interestingly, survival was high and uncorrelated among the three tested feeding frequencies. In temperate regions of the world, it is often necessary to overwinter fish so that they reach market-size the following growing season. Moffett and Hunt (1945) noted that bluegill in Cedar Lake, Michigan, consumed very little during the winter. Channel catfish (*Ictalurus punctatus*) lost weight during the winter when either unfed (Kim and Lovell, 1995), minimally fed (Nanninga et al., 2011), or fed according to a temperature-based feeding chart (Webster et al., 1994). In contrast, Webster et al. (1992) observed slight increases in weight gain (5.4%) when catfish were fed according to Robinette et al. (1982) during the winter. The lack of mortality observed in our study may be related to differences between temperature-controlled tank studies as opposed to fish overwintered in earthen ponds subjected to a variety of additional stressors. Stressors in pond scenarios include, among others, being chased and preyed upon by fish eating birds or other mammals, water quality issues, and temperature fluctuations. These are potentially confounding factors that we sought to control when investigating feeding frequency as a potential primary factor controlling winter mortality, but the absence of these additional stressors may have contributed to high survival rates in our controlled study,

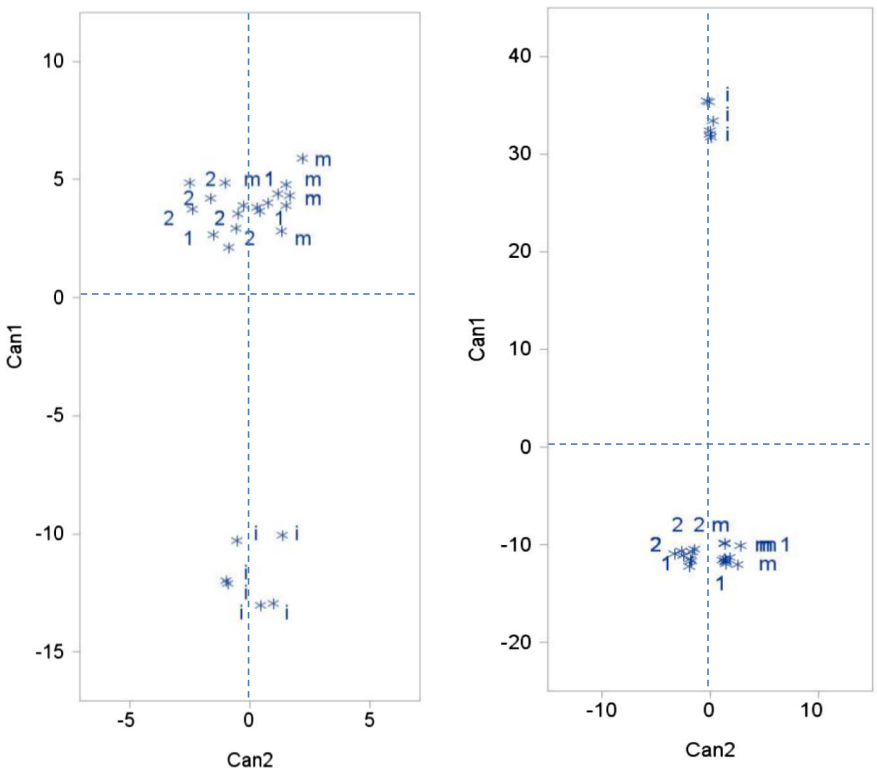

Fig. 1. Scatter plots of the first two canonical variates (Can1, Can2) from the analysis of fatty acid profiles in initial and fed native bluegill (left panel) and hybrid bluegill (right panel) held at 7–9 °C for 13 weeks. Symbols are for initial fish (i) or fish fed once per month (m), once per week (1), or twice per week (2).

Table 5
Class means and fatty acid loading scores of the first two canonical variates (*Can1*, *Can2*) for initial vs. fed native and hybrid bluegill held at 7–9 °C for 13 weeks.[1] Fish were fed once per month (m), once per week (1), or twice per week (2).

	Native		Hybrid	
	Can1	*Can2*	*Can1*	*Can2*
	Class means		*Class means*	
Initial (i)	− 11.73	0.07	28.13	0.91
1 ×/month (m)	4.43	1.20	− 6.61	− 5.07
1 ×/week (1)	3.74	0.14	− 9.62	− 4.15
2 ×/week (2)	3.56	− 1.41	− 11.90	8.31
Fatty acid[2]	*Loading scores*		*Loading scores*	
14:1	–	–	2.44	− 0.05
16:1	0.94	0.36	− 0.87	− 1.48
18:0	− 0.49	0.30	1.40	− 0.68
19:0	–	–	4.53	− 0.72
20:0	–	–	− 3.05	2.22
20:2	–	–	2.18	− 8.52
20:3	–	–	− 2.92	6.07
20:4 (ARA)	–	–	− 5.70	− 3.37
20:5 (EPA)	1.34	0.92	–	–
22:5 (DPA)	–	–	3.32	4.35
Eigenvalue	*55.14*	*1.04*	*320.7*	*33.83*
Variance	*0.98*	*0.02*	*0.90*	*0.09*
Cum. *Var*[3]	*0.98*	*1.00*	*0.90*	*0.99*
R^2	*0.982*	*0.509*	*0.997*	*0.971*

[1]Class means locate the center of each group of fatty acid profiles in Fig. 1. Loading scores are pooled within-class standardized canonical coefficients; scores with the largest absolute values correspond to fatty acids with the greatest discriminatory ability.
[2]Fatty acid abbreviations as in Table 4.
[3]Cumulative variance.

At least for the duration of this study, bluegill suffered no adverse effects from weight loss but the concentrations and composition of fatty acids (FAs) changed markedly in response to feeding frequency and low temperature, suggesting that perhaps winter mortality in bluegill production is more related to diet composition rather than feeding schedule. One of the primary means that dietary fatty acids affect the metabolism and health of fish is by altering the functioning of the cellular membranes in which they become incorporated leading to changes in circulatory, pulmonary, reproductive, immune system, and stress responses (Verlhac Trichet, 2010; Tocher and Glencross, 2015). Similar to Blanchard et al. (2008), the predominant FAs in both taxa of bluegill were 16:0, 18:1n-9 and 22:6n-3 (DHA), regardless of feeding regime. The FA profile of fish is affected by several factors including the initial body concentrations, the duration of feeding and growth during the feeding period (Blanchard et al., 2008), and temperature, to name a few. Growth at temperatures employed in the current study would be minimal in bluegill (Wyatt et al., 2008). Hence, reductions in feed consumption during winter could also result in less lipid depots available for maintenance energy and other functions. Presumably, reduced feeding and intermittent starvation during winter would result in mobilization of extrahepatic lipid depots to meet energy needs (Jezierska et al., 1982), and microarray gene expression has confirmed that lipolysis in the muscle of trout is not altered by starvation (Salem et al., 2006).

Although fish FA composition will tend to reflect that of the diet in the fully fed state, other factors also come into play. For example, some FAs appear to differ in their suitability as energy substrates, while others are selectively retained due to their functional properties (Turchini et al., 2011; Sissener et al., 2016), particularly for their critical roles in membrane fluidity, ionic balance, the inflammatory response, disease resistance and immunity (Castell et al., 1972; Tacon, 1996; Glencross, 2009). The observed decrease in saturates in both taxa post-winter, as well as monoenes in post-winter hybrid bluegill, most likely reflect preferential β-oxidation of saturates and monounsaturates for energy (Bell et al., 2003). We did not observe a decrease in most monoenes in post-winter native bluegill compared to initial samples as was suggested by the work of Henderson (1996) and Smith et al. (2003) in regard to starvation; instead, slight increases were seen in some monounsaturates. The work of Farkas et al. (2001) in rainbow trout suggests that native bluegill cell membranes may also selectively incorporate monoenes, in addition to PUFA, as part of the process of adapting to cold temperatures. The one exception was that 18:1n-9

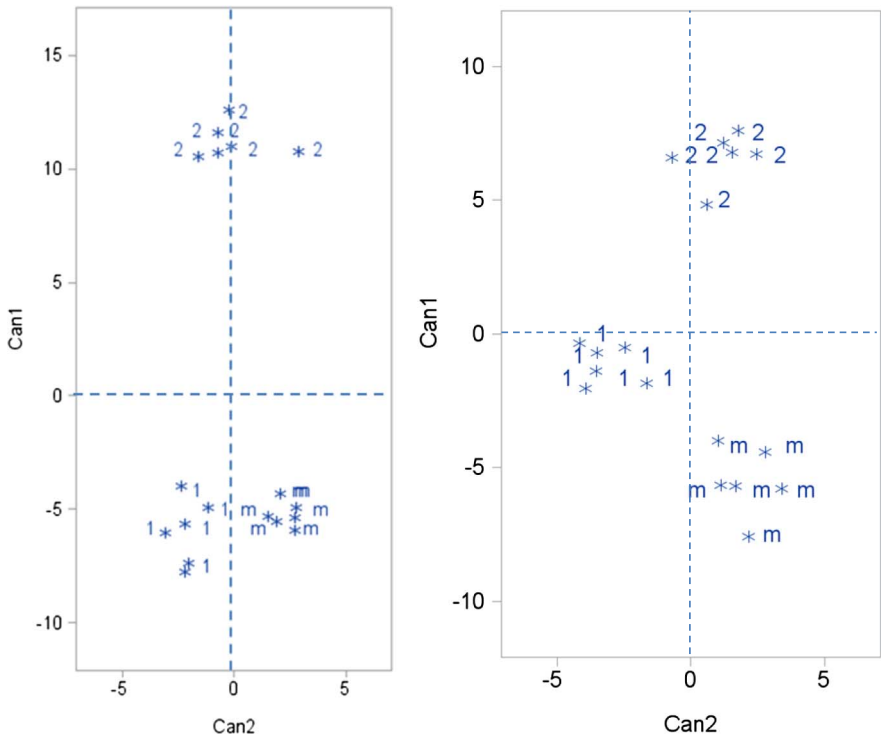

Fig. 2. Scatter plots of the first two canonical variates (Can1, Can2) from the analysis of fatty acid profiles in native bluegill (left panel) and hybrid bluegill (right panel) fed once per month (m), once per week (1), or twice per week (2) at 7–9 °C for 13 weeks.

Table 6
Class means and fatty acid loading scores of the first two canonical variates (*Can1*, *Can2*) for native and hybrid bluegill fed once per month (m), once per week (1), or twice per week (2) at 7–9 °C for 13 weeks.[1]

	Native		Hybrid	
	Can1	*Can2*	*Can1*	*Can2*
	Class means		*Class means*	
1 ×/month (m)	−5.24	2.28	−5.50	2.04
1 ×/week (1)	−5.96	−2.18	−1.12	−3.20
2 ×/week (2)	11.20	−0.10	6.62	1.16
Fatty acid[2]	*Loading scores*		*Loading scores*	
14:0	–	–	−0.42	2.85
15:0	2.48	−2.62	–	–
16:0	6.10	1.59	–	–
16:1	−5.14	2.15	–	–
18:0	−1.73	−0.81	–	–
18:1n-7	−9.15	−2.88	–	–
18:1n-9	10.22	0.99	–	–
18:2	−0.01	0.76	−1.39	−2.15
19:0	–	–	−4.32	−3.15
20:0	–	–	2.41	2.13
20:1	8.05	8.53	–	–
20:4 (ARA)	12.81	9.58	–	–
20:5 (EPA)	−5.31	0.43	−1.57	2.61
22:4	−12.50	−3.81	–	–
22:5 (DPA)	22.74	−4.40	–	–
22:6 (DHA)	−19.29	−3.72	–	–
Eigenvalue	*75.33*	*3.99*	*30.15*	*6.28*
Variance	*0.95*	*0.05*	*0.83*	*0.17*
Cum. *Var*[3]	*0.95*	*1.00*	*0.83*	*1.00*
R^2	*0.987*	*0.800*	*0.968*	*0.863*

[1]Class means locate the center of each group of fatty acid profiles in Fig. 2. Loading scores are pooled within-class standardized canonical coefficients; scores with the largest absolute values correspond to fatty acids with the greatest discriminatory ability.
[2]Fatty acid abbreviations as in Table 4.
[3]Cumulative variance.

concentrations in native bluegill—highest among the monoenes in the initial fish—did decrease from initial levels as feeding frequency decreased at cold temperature; this is consistent with the preferential oxidation of this particular monoene in rainbow trout (Henderson and Sargent, 1985) and the fact that fatty acids provided in surplus (> 15% in our diet) are preferentially oxidized for energy (Tocher, 2003; Turchini et al., 2011). Alternatively, 18:1n-9 can serve as a substrate for LC-PUFA synthesis in the absence of dietary PUFA, with 20:3n-9 being produced (Tocher et al., 1998), but the nearly flat and then decrease in 20:3 concentrations at the lowest feeding frequency compared to initial fish suggests oxidation of 18:1n-9 for energy, rather than partitioning toward synthesis. The declining numerical trends seen in 22:1, from initial fish concentrations to less than half (36% to 0.14%) in native bluegill and less than a tenth in hybrid bluegill (1.01% to 0.09%) fed the least frequently (1 ×/month) are also consistent with the preferential oxidation of this fatty acid irrespective of dietary concentration (Sargent et al., 2002).

Other trends in fatty acid profiles among the initial vs. post-winter bluegills fed at different frequencies are indicative of severe deficiencies in n-6 and n-3 EFAs and preservation of LC-PUFA, especially when fish were fed less than twice per week. In contrast to marine fish, freshwater fish are more able to synthesize LC-PUFA from 18:2 and 18:3 precursors. Since all linoleic acid (LA; 18:2n-6) and alpha linolenic acid (ALA; 18:3n-3) originate in plants and must be diet-derived in fish, dietary intake (however minimal) and initial body depots of LA and ALA in over-wintered bluegill would effectively set the upper limit of total availability. Since LA appears to be selectively retained and resistant to wash out in tilapia (Ng and Chong, 2004), turbot (Regost et al., 2003), and salmon (Bell et al., 2003), the depletion of 18:2 observed in our post-winter native bluegill and minimally fed (once per month; OPM) hybrid bluegill, along with the decrease in ALA in our post-winter hybrid bluegill, support the conclusion of preservation of LC-PUFA in overwintered fish. Linoleic acid is used to synthesize longer chain n-6 fatty acids that are required to conserve cellular eicosanoid signaling and membrane integrity, which are essential during winter (Hochachka and Mommsen, 1995). Therefore, we might have predicted the increases in n-6 intermediates, like 20:2 and 20:3, to ARA (20:4), as well as in ARA itself, which were seen in both species when fed less than twice per week. ARA is the primary precursor for eicosanoids that

function as short-lived autocrine hormones with a wide range of physiological actions, including the control and regulation of immune and inflammatory responses (Castell et al., 1994; Schmitz and Ecker, 2008). Although inflammation is an essential protective response to injury or infection, excessive inflammation due to excessive ARA derived eicosanoids, also leads to acute and chronic pathologies (Montero and Izquierdo, 2010). On the other hand, we also noted an accumulation of dihomo-gamma-linolenic acid, or 8,11,14-eicosatrienoic acid (20:3n-6), the elongation product of gamma-linolenic acid (GLA; 18:3n-6). Dihomo-gamma-linolenic acid competes with ARA for COX and lipoxygenase, inhibiting the production of arachadonic acid's eicosanoids leading to the opposite, i.e., anti-inflammatory, prostaglandin series (Montero and Izquierdo, 2010). Increases in 22:4n-6 levels—an intermediate to 22:5n-6—in both taxa of decreasingly fed overwintered bluegill further substantiate conservation of n-6 LC-PUFA. Overall, n-6/n-3 ratios tended to decline slightly from initial fish as feeding frequency decreased. Increases in n-6/n-3 ratio have been associated with exacerbation of autoimmune and inflammatory disorders in fish (Calder, 2001; Montero et al., 2003) and interestingly, we did not see evidence of inflammatory disorders.

Conservation of n-3 LC-PUFA is a recognized strategy in freshwater fish for coping with LC-PUFA restricted dietary intake (Turchini et al., 2011; Codabaccus et al., 2012) as well as in adaptation to cold (Tocher, 2003). Although it remains to be demonstrated in bluegill, the pathway whereby ALA is converted to 22:6n-3 in rainbow trout is also repressed or induced in the presence or absence, respectively, of diet sources containing 20:5n-3 and 22:6n-3 (Buzzi et al., 1996). Therefore, it is reasonable to hypothesize a similar mechanism operating in post-winter, minimally-fed bluegill; deprived of sufficient LC-PUFA, they will induce synthesis of n-3 LC-PUFA in order to preserve cellular fluidity and integrity, and thus reduce depots of n-3 LC-PUFA precursors relative to initial fish. Additionally, a large demand for 22:6n-3 exists in small, juvenile fish, like those in the current study (< 2 g), particularly for brain and eye development (Bell et al., 2001), which also may have influenced the above trend. Previous work in salmon has demonstrated that conversion of ALA to n-3 LC-PUFA is synergistically stimulated by conversion of LA to n-6 LC-PUFA (Sissener et al., 2016). As previously discussed, the trends seen in the n-6 series fatty acids of overwintered bluegill in the current study are consistent with that observation.

On the other hand, recent work on global gene expression in starved rainbow trout with respect to lipid metabolism suggest that metabolic energy costs are reduced during starvation by slowing down mechanisms of lipid and fatty acid synthesis, lipid binding and transportation, and adipocyte differentiation (Salem et al., 2007). For example, a significant decrease in mRNA accumulation of the fatty acid desaturase 2 gene (FADS2) was observed in starved fish suggesting an advantage to delaying synthesis of unsaturated fatty acids—a considerable energetic cost—until feeding is resumed (Salem et al., 2007). Since fish in the current study were fed, albeit at reduced rates relative to the warm-weather, full-fed state, it is uncertain what role, if any, downregulation of LC-PUFA synthesis machinery played in the current results.

In addition to preservation of n-3 and n-6 PUFA, overwintered bluegill showed increases in some n-9 PUFA. Rats reared on fat-deficient diets have shown a typical increase in 5,8,11-eicosatrienoic acid (20:3n-9; Mead's acid) and 7,10,13-docosatrienoic acid (DTA, 22:3n-9), an isomer from the oleic family (Nervi and Brenner, 1965; Kunau and Couzens, 1971). Docosatrienoic acid has been identified in fish lipids (Richardson et al., 1962; Brenner et al., 1963) and is specifically bound to the β carbon of phosphatidylcholine and phosphatidylethanol amine. Normally, essential fatty acids displace DTA from phospholipids very easily, but in the absence of sufficient EFA, these intermediates may accumulate (Stoll and Spector, 1995).

Seasonal fluctuations in PUFA depots, which may be related to variations in intake, are also evident in the literature. Gokce et al. (2004) reported, for example, that EPA varied between 3.36% (February) to 4.26% (April) during the year in common sole, *Solea solea,* while DHA varied from a low of 16.8% (February) to a high of 20.2% (August). Other fatty acids, such as arachidonic acid (20:4n-6; ARA) varied more broadly; from 12.3% in February to a low of 4.7% in August. On the other hand, some fatty acids changed little in sole fillet throughout the year. Similarly, Nile tilapia (*Oreochromis niloticus*) fed purified diets containing either menhaden oil or coconut oil had minimal differences in whole-body fatty acid compositions when subjected to low temperatures (Atwood et al., 2003). It has been reported that levels of EPA and DHA in wild-caught Indian carp (*Labeo rohita*) decrease in the summer compared to winter (Memon et al., 2010). While this change might be related to physiological aspects associated with the lower water temperatures in winter, the decrease may also be due to fish re-distributing essential fatty acids to gametes used during spring spawning; however, that mechanism would not be expected to influence results of the current study which utilized extremely small (< 2 g) immature juveniles.

In conclusion, significant weight loss and reductions in key fatty acids needed for energetic needs were observed in both native and hybrid bluegill, regardless of feeding regime, indicating that feeding at rates examined in this study did not prove beneficial at 7–9 °C in preserving winter robustness. Additionally, while this experiment evaluated different feeding regimes at one constant low temperature, other external factors such as diet composition, avoiding predation (e.g., by fish-eating migratory waterfowl) and other stressful water quality conditions besides temperature were not investigated. The commercial diet fed in the current study is one typically used in Arkansas sportfish culture, however, total dietary lipid (6%) and the ratio of n − 6:n − 3 fatty acids were rather low and perhaps suboptimal for winter feeding of Centrarchids. In a typical southeastern U.S. winter, water temperature fluctuations are common and they can reach much lower and higher temperatures than were examined in this study. One strategy that may be beneficial for commercial producers is to fatten fish (particularly with lipid rich diets) during the fall months so they have sufficient energy for the winter when feeding will be less frequent. However, further research is needed to examine altered dietary protein to energy ratios at pre-winter, winter, and post-winter (recovery) periods in conjunction with dietary n-6: n-3 and DHA:EPA ratio to determine the optimum concentrations needed to maximize n-3 LC-PUFA deposition and recovery of Centrarchid fish after winter.

Acknowledgments

The authors would like to acknowledge the Arkansas Department of Agriculture and USDA/ARS project number 6028-31630-008-00D for funds supporting this research. The authors would also like to thank the following: Scott Jones, Lin Xie, Herbert Quintero for providing helpful suggestions to an earlier version of the manuscript; Sathyanand Kumaran for technical support; Sonya Lockett for assistance in finding articles referenced in this paper; USDA/ARS lab personnel Rebecca Roberts for fatty acid analyses and Paxton Harper and Chris Scheiderer for sample preparation. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

References

Atwood, H.L., Tomasso, J.R., Webb, K., Gatlin, D.M., 2003. Low temperature tolerance of Nile tilapia, *Oreochromis niloticus*: effects of environmental and dietary factors. Aquac. Res. 34, 241–251.

Bell, M.V., Dick, J.R., Porter, A.E.A., 2001. Biosynthesis and tissue deposition of docosahexaenoic acid (22 : 6n − 3) in rainbow trout (*Oncorhynchus mykiss*). Lipids 36 (10), 1153–1159.

Bell, J.G., McGhee, F., Campbell, P.J., Sargent, J.R., 2003. Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh

fatty acid composition and effectiveness of subsequent fish oil "wash out". Aquaculture 218 (1), 515–528.
Blanchard, G., Makombu, J.G., Kestemont, P., 2008. Influence of different dietary 18: 3n-3/18: 2n-6 ratio on growth performance, fatty acid composition and hepatic ultrastructure in Eurasian perch, *Perca fluviatilis*. Aquaculture 284 (1), 144–150.
Brenner, R.R., Vazza, D.V., De Tomás, M.E., 1963. Effect of a fat-free diet and of different dietary fatty acids (palmitate, oleate, and linoleate) on the fatty acid composition of fresh-water fish lipids. J. Lipid Res. 4 (3), 341–345.
Brunson, M.W., Robinette, H.R., 1986. Evaluation of male bluegill × female green sunfish hybrids for stocking Mississippi farm ponds. North Am. J. Fish. Manag. 6, 156–167.
Buzzi, M., Henderson, R.J., Sargent, J.R., 1996. The desaturation and elongation of linolenic acid and eicosapentaenoic acid by hepatocytes and liver microsomes from rainbow trout (*Oncorhynchus mykiss*) fed diets containing fish oil or olive oil. Biochim. Biophys. Acta 1299, 235–244.
Calder, P.C., 2001. Polyunsaturated fatty acids, inflammation, and immunity. Lipids 36, 1007–1024.
Castell, J.D., Sinnhuber, R.O., Wales, J.H., Lee, J.D., 1972. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): physiological symptoms of EFA deficiency. J. Nutr. 102, 87–92.
Castell, J.D., Bell, J.G., Tocher, D.R., Sargent, J.R., 1994. Effects of purified diets containing different combination arachidonic and docosahexaenoic acid on survival, growth and fatty acid composition of juvenile turbot (*Scophthalmus maximus*). Aquaculture 128, 315–333.
Codabaccus, B.M., Carter, C.G., Bridle, A.R., Nichols, P.D., 2012. The "n − 3 LC-PUFA sparing effect" of modified dietary n − 3 LC-PUFA content and DHA to EPA ratio in Atlantic salmon smolt. Aquaculture 356, 135–140.
Farkas, T., Fodor, E., Kitajka, K., Halver, J.E., 2001. Response of fish membranes to environmental temperature. Aqua. Res. 32, 645–655.
Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.
Fontaine, L.P., Whiteman, K.W., Li, P., Burr, G.S., Webb, K.A., Goff, J., Gatlin III, D.M., Neill, W.H., Davis, K.B., Vega, R.P., 2007. Effects of temperature and feed, energy on the performance of juvenile red drum. T. Am. Fish. Soc. 136, 1193–1205.
Glass, R.L., Krick, T.P., Eckharot, A.E., 1974. New series of fatty acids in northern pike (*Esox lucius*). Lipids 9 (12), 1004–1008.
Glencross, B.D., 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev. Aquacult. 1, 71–124.
Gokce, M.A., Tasbozan, O., Celik, M., Tabakoglu, S.S., 2004. Seasonal variations in proximate and fatty acid compositions of female common sole (*Solea solea*). Food Chem. 88, 419–423.
Goodgame-Tiu, L.S., Webster, C.D., Tidwell, J.H., Reed, E.B., 1994. Overwintering channel catfish, *Ictalurus punctatus*, and blue catfish, *I. furcatus*, in cages. Trans. Kent. Acad. Sci. 55, 127–130.
Hair, J.F., Anderson, R.E., Tatham, R.L., Black, W.C., 2005. Multivariate data analysis, 6th edition. Prentice Hall, Englewood Cliffs, New Jersey, USA.
Hazel, J.R., Livermore, R.C., 1990. Fatty-acyl coenzyme - a pool in liver if rainbow trout (*Salmo gairdneri*): effects of temperature-acclimation. J. Exp. Zool. 256, 31–37.
Henderson, R.J., 1996. Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. Arch. Anim. Nutr. 49, 5–22.
Henderson, R.J., Sargent, J.R., 1985. Chain length specificities of mitochondrial and peroxisomal β-oxidation of fatty acids in rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 82B, 79–85.
Hicks, C.E., Ellersieck, M.R., Borgwarddt, C.J., 2009. Production methods for food-sized bluegills. North Am. J. Aquacult. 71, 52–58.
Hoagland, R.H., Davis, D.A., Tuan, N.A., McGraw, W.J., 2003. Evaluation of practical bluegill diets with varying protein and energy levels. North Am. J. Aquacult. 65, 147–150.
Hochachka, P.W., Mommsen, T.P., 1995. Biochemistry and Molecular Biology of Fishes, Environmental and Ecological Biochemistry. Vol. 5 Elsevier Press, Amsterdam, the Netherlands.
Jezierska, B., Hazel, J.R., Gerking, S.D., 1982. Lipid mobilization during starvation in the rainbow trout, *Salmo gairdneri* Richardson, with attention to fatty acids. J. Fish Biol. 21, 681–692.
Johnson, R.A., Wichern, D.W., 2002. Applied Multivariate Statistical Data Analysis, 5th edition. Prentice Hall, Englewood Cliffs, New Jersey, USA.
Kelly, P.B., Reiser, R., Hood, D.W., 1958. The effect of diet on fatty acid composition of several species of freshwater fish. J. Amer. Oil Chem. Soc. 35 (10), 501–505.
Kim, M.K., Lovell, R.T., 1995. Effect of overwinter feeding regimen on body weight, body composition and resistance to *Edwardsiella ictaluri* in channel catfish, *Ictalurus punctatus*. Aquaculture 134, 237–246.
Kunau, W.-H., Couzens, B., 1971. Studies on the partial degradation of polyunsaturated fatty acids in subcellular fractions of rat liver. Hoppe-Seyler's Zeitschrift für physiologische Chemie. 352 (2), 1297–1305.
Lemke, A.E., 1977. Optimum temperature for growth of juvenile bluegills. Prog. Fish-Cult. 39 (2), 55–57.
Masagounder, K., Hayward, R.S., Firman, J., 2011. Comparison of dietary essential amino acid requirements determined from group- versus individually-housed juvenile bluegill, *Lepomis macrochirus*. Aquac. Nutr. 17, e559–e571.
McNulty, E., Stone, N., Lochmann, S., 2000. Effect of winter feeding rate, feed form and trigger temperature on yield and condition of golden shiners, *Notemigonus crysoleucas*. J. Appl. Aquac. 10 (3), 69–82.
Memon, N.N., Talpur, F.N., Bhanger, M.I., 2010. Nutritional aspects and seasonal influence on fatty acid composition of carp (*Labeo rohita*) from the Indus River, Pakistan. Pol. J. Food Nutr. Sci. 60 (3), 217–223.
Moffett, J.W., Hunt, B.P., 1945. Winter feeding habits of bluegills, *Lepomis macrochirus* Rafinesque, and yellow perch, *Perca flavescens* (Mitchill) in Cedar Lake, Washtenaw County. Michigan. T. Am. Fish Soc. 73 (1), 231–242.
Montero, D., Izquierdo, M., 2010. Welfare and health of fish fed vegetable oils as alternative lipid sources to fish oil. In: Turchini, G.M., Ng, W.-K., Tocher, D.R. (Eds.), Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. Taylor & Francis, CRC Press, Boca Raton, USA, pp. 439–485.
Montero, D., Kalinowski, T., Obach, A., Robaina, L., Tort, L., Caballero, M.J., Izquierdo, M.S., 2003. Vegetable lipid sources for gilthead seabream (*Sparus aurata*): effects on fish health. Aquaculture 225, 353–370.
Nanninga, A.S., Engle, C.R., Stone, N., Goodwin, A.E., 2011. Effects of winter feeding of channel catfish on production in multibatch systems. North Am. J. Aquacult. 73, 60–67.
Nervi, A.M., Brenner, R.R., 1965. Identification and origin of docosatrienoic acid of rat phospholipids. Biocim. Biophys. Acta 106, 205–207.
Ng, W.K., Chong, C.Y., 2004. An overview of lipid nutrition with emphasis on alternative lipid sources in tilapia feeds. In: Bolivar, R.B., Mair, G.C., Fitzsimmons, K. (Eds.), New Dimensions in Farmed Tilapia. Proceedings of the Sixth International Symposium on Tilapia in Aquaculture. American Tilapia Association, Pine Bluff, AR, pp. 241–248. http://cals.arizona.Edu/azaqua/ista/ista6/ista6web/pdf/241.pdf (Last accessed 09/21/2016).
Ott, R.L., 1977. An Introduction to Statistical Methods and Data Analysis. Duxbery Press, North Scituate, MA.
Regost, C., Arzel, J., Robin, J., Rosenlund, G., Kaushik, S.J., 2003. Total replacement of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*): 1. Growth performance, flesh fatty acid profile, and lipid metabolism. Aquaculture 217 (1), 465–482.
Richardson, T., Tappel, A.L., Smith, L.M., Houle, C.R., 1962. Polyunsaturated fatty acids in mitochondria. J. Lipid Res. 3 (3), 345–350.
Robinette, H.R., Bush, R.L., Newton, S.H., Heskins, C.J., Davis, S., Stickney, R.R., 1982. Winter feeding of channel catfish in Mississippi, Arkansas and Texas. Proc. Southeast. Assoc. Fish Wildl. Agencies 36, 162–171.
Rowan, M., Stone, N., 1994. Winter feeding of golden shiners: year II. Arkansas Aquafarming 12 (1), 5–6.
Rude, N.P., Trushenski, J.T., Whitledge, G.W., 2016. Fatty acid profiles are biomarkers of fish habitat use in a river-floodplain ecosystem. Hydrobiology 773, 63–75.
Salem, M., Kenney, P.B., Rexroad, C.E., Yao, J., 2006. Molecular characterization of muscle atrophy and proteolysis associated with spawning in rainbow trout. Comp. Biochem. Physiol. D Genomics Proteomics 1 (1), 227–237.
Salem, M., Silverstein, J., Rexroad III, C.E., Yao, J., 2007. Effect of starvation on global gene expression and proteolysis in rainbow trout (*Oncorhynchus mykiss*). BMC Genomics 8 (1), 1–16.
Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition, 3rd edition. Academic Press, San Diego, CA, pp. 181–257.
Schmitz, G., Ecker, J., 2008. The opposing effects of n-3 and n-6 fatty acids. Prog. Lipid Res. 47, 147–155.
Sissener, N.H., Sanden, M., Torstensen, B.E., Waagbø, R., Stubhaug, I., Rosenlund, G., 2016. High dietary 18:2n-6/18:3n-3 ratio does not inhibit elongation and desaturation of 18:3n-3 to EPA and DHA in Atlantic salmon (*Salmo salar* L.). Aquacult. Nutr. http://dx.doi.org/10.1111/anu.12457.
Smith, G.G., Thompson, P.A., Ritar, A.J., Dunstan, G.A., 2003. Effects of starvation and feeding on the fatty acid profiles of stage I phyllosoma of the spiny lobster, *Jasus edwardsii*. Aquac. Res. 34 (5), 419–426.
Stoll, L.L., Spector, A.A., 1995. Functional and ultrastructural effects of essential fatty acid deficiency in kidney epithelial cells. Lipids 30 (12), 1093–1103.
Tacon, A.G.J., 1996. Lipid nutritional pathology in farmed fish. Arch. Anim. Nutr. 49, 33–39.
Tidwell, J.H., Webster, C.D., Clark, J.A., 1992. Growth, feed conversion, and protein utilization of female green sunfish × male bluegill hybrids fed isocaloric diets with different protein levels. Prog. Fish-Cult. 54 (4), 234–239.
Tidwell, J.H., Webster, C.D., 1993. Effects of stocking density and dietary protein on green sunfish (*Lepomis cyanellus*) x bluegill (*L. macrochirus*) hybrids overwintered in ponds. Aquaculture 113, 83–89.
Tidwell, J.H., Webster, C.D., Clark, J.A., Brunson, M.W., 1994. Pond culture of female green sunfish (*Lepomis cyanellus*) × male bluegill (*L. macrochirus*) hybrids stocked at two sizes and densities. Aquaculture 126, 305–313.
Tocher, D.R., 2003. Metabolism and function of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107–184.
Tocher, D.R., Glencross, B.D., 2015. Lipids and fatty acids. In: Lee, C.-S., Lim, C., Gatlin, D.M., Webster, C.D. (Eds.), Dietary Nutrients, Additives and Fish Health, First Edition. John Wiley & Sons, Hoboken, N.J., USA, pp. 47–94.
Tocher, D.R., Leaver, M.J., Hodgson, P.A., 1998. Recent advances in the biochemistry and molecular biology of fatty acyl desaturases. Prog. Lipid Res. 37, 73–117.
Turchini, G.M., Francis, D.S., Senadheera, S.P.S.D., Thanuthong, T., De Silva, S.S., 2011. Fish oil replacement with different vegetable oils in Murray cod: evidence of an "omega-3 sparing effect" by other dietary fatty acids. Aquaculture 315 (3), 250–259.
Twibell, R.G., Wilson, K.A., Sanders, S., Brown, P.B., 2003. Evaluation of experimental and practical diets for bluegill (*Lepomis macrochirus*) and hybrid bluegill (*L. cyanellus* × *L. macrochirus*). J. World Aquacult. Soc. 34, 487–495.
Verlhac Trichet, V., 2010. Nutrition and immunity: an update. Aqua. Res. 41, 356–372.
Wang, H.P., Wallat, G.K., Hayward, R.S., Tiu, L.G., O'Bryant, P., Rapp, D., 2009. Establishment of mostly male groups of bluegills by grading selection and evaluation of their growth performance. North Am. J. Aquacult. 71 (3), 216–223.
Watanabe, T., 1982. Lipid nutrition in fish. Comp. Biochem. Physiol. B 73, 3–15.
Webster, C.D., Tidwell, J.H., Goodgame, L.S., Clark, J.A., Yancey, D.H., 1992. Winter feeding and growth of channel catfish fed diets containing varying percentages of distillers grains with solubles as a total replacement of fish meal. J. Appl. Aquac. 1

(4), 1–14.
Webster, C.D., Wurts, W.A., Tidwell, J.H., Goodgame, L.S., Clark, J.A., Yancey, D.H., 1994. Effects of stocking density on growth of juvenile channel catfish overwintered in ponds. J. Appl. Aquac. 4 (3), 79–87.
Wyatt, T., Lyon, D., Barkoh, A., 2008. Guidelines for the culture of bluegill sunfish, *Lepomis macrochirus*. In: Texas Parks and Wildlife Management Data Series 251. TX, Austin (29 pp).
Yang, M., Wang, J., Han, T., Yang, Y., Li, X., Jiang, Y., 2016. Dietary protein requirement of juvenile bluegill sunfish (*Lepomis macrochirus*). Aquaculture 459, 191–197.